Heredity (2008) 100, 382–389

www.nature.com/hdy

ORIGINAL ARTICLE

Genetics of variation in adiponectin in pedigreed baboons: evidence for pleiotropic effects on adipocyte volume and serum adiponectin

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To detect and localize the effects of genes influencing variation in adiponectin mRNA and protein levels, we conducted statistical genetic analyses of circulating concentrations of adiponectin and adiponectin (ADIPOQ) mRNA expression in omental adipose tissue in adult, pedigreed baboons (*Papio anubis*). An omental adipose tissue biopsy and blood sample were collected from 427 baboons from the colony at the Southwest Foundation for Biomedical Research, San Antonio, TX. Total RNA was isolated from adipose tissue and adiponectin mRNA levels were assayed by real-time, quantitative reverse transcriptase-PCR. Adiponectin, insulin, glucose, cholesterol, high-density lipoproteins and triglycerides were measured in fasting serum. Quantitative genetic analyses were conducted for adiponectin mRNA and serum protein using a maximum likelihood-based variance decomposition approach. A genome-wide linkage analysis was conducted using adiponectin mRNA and protein levels as phenotypes. Significant heritability was estimated for ADIPOQ mRNA levels ($h^2 = 0.19 \pm 0.07$, $P = 0.01$) and protein levels ($h^2 = 0.28 \pm 0.14$, $P = 0.003$). Genetic correlations were found between adiponectin protein and body weight ($\rho_G = -0.51$, $P = 0.03$), cell volume ($\rho_G = -0.73$, $P = 0.04$), serum triglycerides ($\rho_G = -0.67$, $P = 0.03$), and between adiponectin mRNA and glucose ($\rho_G = 0.93$, $P < 0.01$). A logarithm of odds score of 2.9 was found for ADIPOQ mRNA levels on baboon chromosome 4p, which is orthologous to human 6p21. There is a significant genetic component affecting variation in the analyzed traits, and common genes may be influencing adiponectin expression, adipocyte volume, body weight and circulating triglycerides. The region on 6p21 has been linked to diabetes-related phenotypes in human studies.
Heredity (2008) **100,** 382–389; doi:10.1038/sj.hdy.6801089; published online 20 February 2008

Keywords: adiponectin; animal model; adipose tissue; QTL; pleiotropy; baboon

Introduction

The hormone adiponectin is produced by white and brown adipose tissue in humans (Kershaw and Flier, 2004), rodents (Lihn *et al*., 2004) and nonhuman primates (Hotta *et al*., 2001). The adiponectin mRNA transcript (ADIPOQ) is the most abundant in adipose tissue, and the translated product is present in plasma in high concentrations (1% of protein in human plasma) (Maeda *et al*., 1996; Kershaw and Flier, 2004). Adiponectin has been consistently associated with regulation of insulin sensitivity, lipid metabolism, inflammation and the risk for the development of atherosclerosis (Fruebis *et al*., 2001; Matsuda *et al*., 2002; Shimada *et al*., 2004). Circulating adiponectin levels are low in obesity and inflammation (Bruun *et al*., 2003; Fernández Real *et al*., 2003), insulin resistance (Silha *et al*., 2003; Nakamura *et al*., 2004) and type 2 diabetes (Weyer *et al*., 2001), and higher concentrations of this protein are associated with improvement of metabolic abnormalities (Esposito *et al*., 2003).

Previous investigations have reported obesity and metabolic abnormalities in baboons, which resemble those observed in human obesity and the metabolic syndrome (Banks *et al*., 2003; Comuzzie *et al*., 2003) and have confirmed the value of this species as an animal model for the study of obesity-related conditions. An earlier study in our laboratory found that adiponectin levels in plasma are inversely correlated with insulin resistance in adult baboons (Tejero *et al*., 2004).

Circulating levels of adiponectin are heritable (Comuzzie *et al*., 2001; Lindsay *et al*., 2003; Butte *et al*., 2005; Pollin *et al*., 2005). Genome-wide scan analyses have identified quantitative trait loci (QTL) linked to variation in adiponectin levels in several geographically and ethnically diverse human populations. Significant linkage with logarithm of odds score (LOD score) >3 has been reported on chromosomes 5, 14 (Comuzzie *et al*., 2001) and 11 (Tejero *et al*., 2007) and signals on chromosomes 10p and 3p have been replicated across different populations (Comuzzie *et al*., 2001; Chuang

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Received 2 April 2007; revised 14 September 2007; accepted 8 November 2007; published online 20 February 2008

et al., 2005; Tejero *et al*., 2007). The present investigation conducted a genome-wide scan using adiponectin mRNA expression levels and circulating protein levels in baboons as quantitative phenotypes and explored the pleiotropy between adiponectin phenotypes and traits associated with the metabolic syndrome.

Methods

Animals

The studied sample consisted of 427 adult baboons from the pedigreed colony at the Southwest National Primate Research Center located at the Southwest Foundation for Biomedical Research at San Antonio, TX, with 130 male and 297 nonpregnant, nonlactating females. All baboons are gang-housed and fed *ad libitum* on a standard low-fat chow diet (Harlan Tecklad 15% Monkey Diet, 8715).

Genotypes

Baboon genomic DNA was isolated from leukocytes using a phenol–chloroform method and amplified using fluorescently labeled, published human PCR primers as described elsewhere (Cox *et al*., 2006). The genotyping procedure in the present study included 330 markers, consisting of short tandem repeat polymorphisms covering the autosomes and spaced at approximately 7.2-cM intervals. Genotypes were analyzed using gel electrophoresis on ABI automated sequencers and Genescan, Genotyper and Gene mapper software (Applied Biosystems, Foster City, CA, USA). Details of the latest baboon short tandem repeat polymorphism map have been previously published (Cox *et al*., 2006).

Phenotypes

All samples were collected after an overnight fast (12 h), with the animals under sedation with ketamine. An 8 ml sample of blood was drawn from the antecubital vein and after clotting all samples were centrifuged for 10 min at 2000 *g* for serum separation. The serum was then decanted and frozen at −80 °C for subsequent analyses of glucose, lipids and proteins. A 1 g biopsy of omental adipose tissue was obtained from all baboons. Biopsies were analyzed immediately for cell volume as described by Lewis *et al*. (1986). The remaining sample was frozen for further extraction of total RNA using Trizol Reagent (Molecular Technology, Gaithersburg, MO, USA). The RNA yield and purity were analyzed by ultraviolet spectrophotometry. Integrity of RNA was determined by electrophoresis in a 1.2% denaturant agarose gel stained with ethidium bromide. All samples were treated with DNAse (Invitrogen, Carisbad, CA, USA) for 15 min at 37 °C to eliminate traces of genomic DNA.

Cloning of a baboon adiponectin cDNA fragment

A cDNA fragment of baboon adiponectin was cloned from total RNA of omental adipose tissue by the two-step methods of reverse transcription followed by a polymerase chain reaction (RT-PCR) method using the THERMOSCRIPT RT-PCR System (Gibco BRL Life Technologies Inc., Gaithersburg, MD, USA). Reverse transcription was conducted with hexamers (Invitrogen). Two microliters of the cDNA product were used for the amplification of the adiponectin transcript. Published primers for the rhesus monkey sequence were used (Hotta *et al*., 2001). The amplified product was cloned using the CloneAmp pAMP1 kit (Gibco BRL Life Technologies Inc.). The cloned baboon adiponectin cDNA fragment was sequenced using an ABI 3100 automated DNA sequencer with Big Dye Terminator kit (Applied Biosystems). The sequenced product was analyzed in BLAST (www.ncbi.nlm.nih.gov) for prediction of the encoded amino acids and alignment with similar sequences.

Quantification of adiponectin mRNA

Adiponectin mRNA expression in omental adipose tissue was measured by real-time, quantitative RT-PCR (Taq Man, Applied Biosystems). The primers and probe sequences were designed with the Primer Express Software Version 1 (Applied Biosystems) using the baboon adiponectin cDNA clone. The sequences of forward and reverse primers were 5′-TCCTCCTGCCT GTCCTGG-3′ and 5′-CGCCCTCCTGAATCTTCTCAT-3′, respectively. The sequence for the adiponectin probe was 5′-TAAACGTGGACCAGGCCTCCGG-3′. Ribosomal 18S RNA (rRNA) was used as an internal control and measured by the Universal 18S system from Ambion (Austin, TX, USA). The primers to competimers ratio was 4:6. The probe for 18S was the rRNA Ambiprobe from Applied Biosystems. A sample of 50 ng of total RNA was used per assay. Data were obtained as C_t values (the number of cycles at which logarithmic plots of PCR product accumulation cross a specific threshold line), according to the manufacturer's specifications (Applied Biosystems) and transformed into number of mRNA copies. Adiponectin expression was corrected for 18S ribosomal mRNA by dividing by the number of copies.

Assays for glucose, insulin, adiponectin protein and lipids

Glucose was analyzed with an Analox spectrophometer. Lipids were assayed by standard enzymatic procedures and insulin and adiponectin were assayed by quimioluminiscence (Linco Research, Lake Charles, MO, USA). Inter and intra-assay variation in all parameters was $<5\%$. Comparison of adiponectin circulating concentrations between male and female baboons was conducted by Student's *t*-test for independent samples.

Analysis of tissue-specific gene expression of baboon adiponectin

Tissues from stomach fundus, ovary, skeletal muscle, omental and subcutaneous adipose tissue, monocytes, pancreas, hypothalamus, small intestine and colon were isolated from a euthanized adult baboon. Placenta was obtained from a full-term baboon pregnancy, after delivery. RNA was isolated from the tissues and the adiponectin transcript was amplified from the samples by two-step RT-PCR as described above. Reverse transcription was conducted as described above. Two microliters of the cDNA product were used for the amplification of the adiponectin transcript with published primers, and 18S ribosomal RNA expression was used as control. Samples were analyzed on a 2% agarose gel stained with ethidium bromide, and the presence of a PCR product of the correct size was considered evidence for tissue-specific adiponectin gene expression.

Statistical genetic analysis

Statistical genetic analyses were conducted using the computer package SOLAR (Almasy and Blangero, 1998), which applies a maximum likelihood-based, variance decomposition method. For this analysis, the phenotypic variance (σ^2_P) was divided into two major components, the additive genetic (σ^2_G) and nongenetic (σ^2_E), or environmental. Heritability can be calculated as the proportion of the trait variance that results from the additive genetic effects ($h^2 = \sigma^2_G/\sigma^2_P$). The likelihood of the model is estimated and compared with the likelihood of a model in which the effect is absent (heritability of zero). The approximately asymptotic distribution is a 1/2:1/2 mixture of a χ^2 variable with one degree of freedom and a point mass at zero. Heritabilities of the studied variables were estimated after accounting for the mean effects of covariates including sex, age, sex-by-age interaction, age^2, sex by age^2 and weight. Significance of the residual heritability estimates was assessed by likelihood ratio test. To investigate shared genetic effects (pleiotropy) between pairs of phenotypes, bivariate quantitative genetic analyses were performed. In these analyses, we obtained maximum likelihood estimates of both additive genetic and environmental correlations (ρ_G and ρ_E, respectively) between adiponectin protein and mRNA levels and the other studied phenotypes. From these estimates, we calculated phenotypic correlations (ρ_P) between trait pairs that accounted for the nonindependence between relatives as:

$$\rho_p = \rho_G\sqrt{h_1^2}\sqrt{h_2^2} + \rho_E\sqrt{(1-h_1^2)}\sqrt{(1-h_2^2)}$$

Genome-wide, multipoint linkage scans were conducted in SOLAR using adiponectin mRNA and protein levels using body weight, sex, age and their interaction as covariates. Empirical LOD score adjustment was conducted by the method described by Blangero *et al*. (2000). To control for the overall false positive rate in whole genome linkage screens, we employed a modification of

```
Homo sapiens (Hs)  ATGCTGTTGCTGGGAGCTGTTCTACTGCTATTAGCTCTGCCCGGTCATGACCAGGAAACC 60
Papio anubis (Pa)  ---ATGTTGCTGGGAGCTGTTCTACTGCTATTAGTTCTGCCCAGTCATGGCCAGGATACC 57
                                                      *       *      *      *

Hs                 ACGACTCAAGGGCCCGGAGTCCTGCTTCCCCTGCCCAAGGGGGCCTGCACAGGTTGGAT 119
Pa                 ACAACTCAAGGGCCGGGAGTCCTGCTTCCTCTGCCCAAGGGGGCCTGCACAGGTTGGAT 117
                     *           *              *

Hs                 GGCGGGCATCCCAGGGCATCCGGGCCATAATGGGGCCCCAGGCCGTGATGGCAGAGATGG 179
Pa                 GGCAGGCATCCCAGGGCATCCAGGCCATAATGGGGTCCCAGGTCGTGATGGCAGAGATGG 177
                      *                 *             *      *

Hs                 CACCCCTGGTGAGAAGGGTGAGAAAGGAGATCCAGGTCTTATTGGTCCTAAGGGAGACAT 239
Pa                 CACCCCTGGCGAGAAGGGTGAGAAAGGAGATCCAGGTCTTATTGGTCCTAAGGGAGACAC 237
                           *


Hs                 CGGTGAAACCGGAGTACCCGGGGCTGAAGGTCCCCGAGGCTTTCCGGGAATCCAAGGCAG 299
Pa                 TGGTGAAACTGGAGTAACCGGGGCTGAAGGTCCCCGAGGCTTTCCGGGAATCCAAGGCAG 296
                   *       *      *

Hs                 GAAAGGAGAACCTGGAGAAGGTGCCTATGTATACCGCTCAGCATTCAGTGTGGGATTGGA 359
Pa                 GAAAGGAGAACCTGGAGAAGGTGCCTATGTATACCGCTCAGCATTCAGTGTGGGATTGGA 356


Hs                 GACTTACGTTACTATCCCCAACATGCCCATTCGCTTTACCAAGATCTTCTACAATCAGCA 419
Pa                 GACCTACGTTACTGTCCCCAACATGCCCATTCGCTTTACCAAGATCTTCTACAATCAGCA 416
                      *         *

Hs                 AAACCACTATGATGGCTCCACTGGTAAATTCCACTGCAACATTCCTGGGCTGTACTACTT 479
Pa                 AAACCACTATGATGGCTCCACTGGTAAATTCCACTGCAACATTCCTGGGCTGTACTACTT 476


Hs                 TGCCTACCACATCACAGTCTATATGAAGGATGTGAAGGTCAGCCTCTTCAAGAAGGACAA 539
Pa                 TGCCTACCACATCACAGTCTATATGAAGGATGTGAAGGTCAGCCTCTTCAAGAAGGACAA 536

Hs                 GGCTATGCTCTTCACCTATGATCAGTACCAGGAAAATAATGTGGACCAGGCCTCCGGCTC 599
Pa                 GGCTATGCTCTTCACCTATGACCAGTACCAGGAAAATAACGTGGACCAGGCCTCCGGCTC 596
                                     *                 *

Hs                 TGTGCTCCTGCATCTGGAGGTGGGCGACCAAGTCTGGCTCCAGGTGTATGGGGAAGGAGA 659
Pa                 TGTGCTCCTGCATCTGGAGGTGGGCGACCAAGTCTGGCTCCAGGTGTATGGGGAAGGAGA 656


Hs                 GCGTAATGGACTCTATGCTGATAATGACAATGACTCCACCTTCACAGGCTTTCTTCTCTA 719
Pa                 GCGTAATGGACTCTATGCTGATAATGACAATGACTCCACCTTCACAGGCTTTCTTCTCTA 711

Hs                 CCA 735
Pa                 CCA 719
```

Figure 1 Comparison between baboon (*Papio anubis*) and human (*Homo sapiens*) adiponectin cDNA sequence. *Indicates difference in nucleotide sequence.

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Hs
MLLLGAVLLLLALPGHDQETTTQGPGVLLPLPKGACTGWMAGIPGHPGHNGAPGRDGRDG 60
Pa
MLLLGAVLLLLVLPSHGQDTTTQGPGVLLPLPKGACTGWMAGIPGHPGHNGVPGRDGRDG 59
              * * *                                   *

Hs
TPGEKGEKGDPGLIGPKGDIGETGVPGAEGPRGFPGIQGRKGEPGEGAYVYRSAFSVGLE 120
Pa
TPGEKGEKGDPGLIGPKGDTGETGVTGAEGPRGFPGIQGRKGEPGEGAYVYRSAFSVGLE 119
                    *     *

Hs
TYVTIPNMPIRFTKIFYNQQNHYDGSTGKFHCNIPGLYYFAYHITVYMKDVKVSLFKKDK 180
Pa
TYVTVPNMPIRFTKIFYNQQNHYDGSTGKFHCNIPGLYYFAYHITVYMKDVKVSLFKKDK 179
    *

Hs
AMLFTYDQYQENNVDQASGSVLLHLEVGDQVWLQVYGEGERNGLYADNDNDSTFTGFLLY 240
Pa
AMLFTYDQYQENNVDQASGSVLLHLEVGDQVWLQVYGEGERNGLYADNDNDSTFTGFLLY
```

Figure 2 Adiponectin amino-acid sequences of *Homo sapiens* and *Papio anubis*. * Indicates difference in amino acid sequence.

Table 1 Characteristics of the studied baboons

Trait	*Mean* ± *s.d.* *Male* (n = *130)*	*Mean* ± *s.d.* *Female* (n = *297)*	h^2 ± *s.e.*	P
Age (years)	12.1 ± 3.9	15.9 ± 4.9		
Weight (kg)	31.5 ± 4.5	19.5 ± 4.0	0.79 ± 0.10	0.001
Glucose (mg per 100 ml)	79.5 ± 1.16	82.4 ± 1.2	0.27 ± 0.10	0.0002
Insulin (μIU per 100 ml)	23.4 ± 0.02	42.6 ± 0.06	0.12 ± 0.09	0.057
Cholesterol (mg per 100 ml)	92.9 ± 23.5	121.8 ± 33.8	0.67 ± 0.10	1.4×10^{-19}
HDL-C (mg per 100 ml)	50.6 ± 11.9	53.9 ± 13.3	0.78 ± 0.11	6.8×10^{-19}
Triglycerides (mg per 100 ml)	44.6 ± 1.4	62.5 ± 1.4	0.32 ± 0.11	0.0005
Adiponectin mRNA/18S	21.2 ± 2.3	20.7 ± 2.5	0.19 ± 0.07	0.01
Adiponectin (μg ml^{-1})	10.3 ± 0.14	11.8 ± 0.12	0.28 ± 0.14	0.003
Cell volume (nl)	0.39 ± 0.4	0.58 ± 0.42	0.20 ± 0.12	0.02

Abbreviation: HDL-C, high-density lipoprotein cholesterol.

Table 2 Relative pairs

	427	Self
	121	Parent–offspring
	255	Siblings
	2	Grandparent–grandchild
	43	Avuncular
	2842	Half siblings
	566	Half avuncular
	3	First cousins
	19	Half first cousins
	1	Half sibling and first cousin
	51	Half siblings and first cousins
	7	Half sibling and half avuncular
Total	4337	

an approach advanced by Feingold *et al*. (1993) that takes into account the finite marker density in the linkage map utilized and (as an indicator of pedigree complexity and size) the estimated mean recombination rate in the baboon pedigrees. In the current data set, the thresholds for significant (at $\alpha = 0.05$) and suggestive linkage on a genome-wide basis are LOD = 2.75 and LOD = 1.53, respectively.

Variables were approximately normalized by log transformation before analyses. The selection of the positional candidate genes was conducted using the NCBI database.

Results

The baboon adiponectin cDNA sequence spans 695 bp and is 97% identical to human and 100% identical to the rhesus monkey sequence. There are differences in 7 of the 240 predicted amino acids between human and baboon adiponectin (Figures 1 and 2). The adiponectin transcript was found in omental and subcutaneous adipose tissue. No other tissue had detectable levels of expression of this gene (Figure 4).

The profile of the studied animals is shown in Table 1. Adiponectin levels in serum were significantly higher in female baboons ($P < 0.05$) but no difference by sex was found in levels of adiponectin mRNA expression.

The distribution of the relative pairs in the present study is shown in Table 2. The univariate analysis estimated a significant heritability of 128% for levels of circulating adiponectin protein and 19% for adiponectin mRNA levels in omental adipose tissue using sex, age, age^2 and their interactions as covariates. As observed in Table 1, all the analyzed traits were significantly heritable.

The genetic and environmental correlations between adiponectin protein and mRNA levels and the other analyzed variables are shown in Tables 3 and 4. Significant phenotypic correlations were observed between adiponectin protein and body weight and cell volume. Genetic correlations were significant between adiponectin protein and triglyceride levels, cell volume and body weight. Adiponectin mRNA had significant phenotypic correlations with body weight and significant genetic correlations with glucose levels.

Genome-wide scans were conducted using adiponectin mRNA abundance and protein as quantitative traits. As observed in Figures 3a and b, an LOD score of 2.9 (genome-wide $P = 0.033$) was found on baboon chromosome 4p, which is orthologous to human 6p21, for adiponectin mRNA levels from omental adipose tissue (Figure 4). Figures 3a and b show string plots organized by human orthologous regions. Figure 3c shows the lack of overlap on chromosome 6p21 between the signals for adiponectin protein and mRNA levels. The highest evidence for a QTL influencing circulating levels of adiponectin protein in this investigation was found on human chromosome 4 (LOD score = 1.1).

Discussion

Adiponectin sequence is highly similar to human at the cDNA and amino-acid level. As expected, adiponectin mRNA expression was found in omental and subcutaneous adipose tissue; however, no expression was observed in baboon placenta. Expression and release of adiponectin protein from human placenta has been reported and it is believed to play a role in insulin resistance during pregnancy (Chen *et al*., 2006). The lack of expression of adiponectin in the baboon placenta remains to be explored.

The circulating levels of adiponectin in baboon are similar to those reported for human with females having higher levels of this protein. Heritability of adiponectin protein in serum and the other analyzed traits resemble values in previous human studies and in baboons (Cai *et al*., 2004), indicating a significant genetic contribution to the variance of each of these traits.

The bivariate analyses revealed significant genetic correlations, suggesting the presence of pleiotropy between some of the analyzed phenotype pairs. A highly significant genetic correlation was found between adiponectin mRNA in omental adipose tissue and circulating protein levels indicating that 60.8% of the additive genetic variation in these two phenotypes, as expected, is attributable to the effects of the same gene or genes.

Negative phenotypic correlations between adiponectin protein and cell volume, and body weight indicate the inverse covariation between these phenotypes. This is the first study, to our knowledge, of pleiotropy between adipose cell volume and adiponectin expression levels. The present results indicate the presence of a significant genetic component and suggest the exertion of pleiotropic effects on adiponectin protein, cell volume, body weight and triglycerides.

Adiponectin mRNA levels and fasting glucose levels have shared genetic effects. According to these results, common genes are influencing these phenotypes in an inverse manner. These observations are concordant with findings in previous human and rodent studies showing

Table 3 Phenotypic (ρ_P), genetic (ρ_G) and environmental (ρ_E) correlations ± s.e. between adiponectin protein and the analyzed variables

Trait	ρ_P	P	ρ_G	P	ρ_E	P
Glucose	0.22	0.92	0.31 ± 0.12	0.12	0.19 ± 0.12	0.12
Insulin	−0.15	0.06	−0.09 ± 0.67	0.87	−0.17 ± 0.12	0.12
Cholesterol	0.02	0.26	−0.16 ± 0.24	0.49	0.31 ± 0.16	0.05
Triglycerides	−0.17	0.39	**−0.67** ± 0.29	**0.03**	0.21 ± 0.16	0.15
HDL	0.06	0.24	0.03 ± 0.26	0.90	0.20 ± 0.20	0.34
Cell volume	**−0.24**	**0.00003**	**−0.73** ± 0.34	**0.04**	−0.08 ± 0.13	0.55
Adiponectin mRNA	−0.07	0.32	**0.78** ± 0.27	**0.03**	0.11 ± 0.11	0.39
Body weight	**−0.22**	**0.004**	**−0.51** ± 0.37	**0.03**	0.007 ± 0.12	0.97

Abbreviation: HDL, high-density lipoproteins.
Statistically significant values are in bold.

Table 4 Phenotypic (ρ_P), genetic (ρ_G) and environmental (ρ_E) correlations ± s.e. between adiponectin mRNA in omental adipose tissue and the analyzed variables

Trait	ρ_P	P	ρ_G	P	ρ_E	P
Glucose	0.01	0.65	**−0.50** ± 0.40	**0.04**	0.15 ± 0.09	0.12
Insulin	0.01	0.62	−0.16 ± 0.70	0.82	0.04 ± 0.09	0.62
Cholesterol	0.09	0.10	0.19 ± 0.27	0.46	0.04 ± 0.72	0.72
Triglycerides	0.07	0.60	0.06 ± 0.36	0.88	−0.07 ± 0.11	0.55
HDL	0.09	0.09	0.16 ± 0.26	0.53	0.07 ± 0.15	0.65
Cell volume	−0.16	0.43	−0.90 ± 0.65	0.80	0.01 ± 0.09	0.70
Body weight	**−0.14**	**0.02**	−0.38 ± 0.15	0.14	0.05 ± 0.12	0.86

Abbreviation: HDL, high-density lipoproteins.
Statistically significant values are in bold.

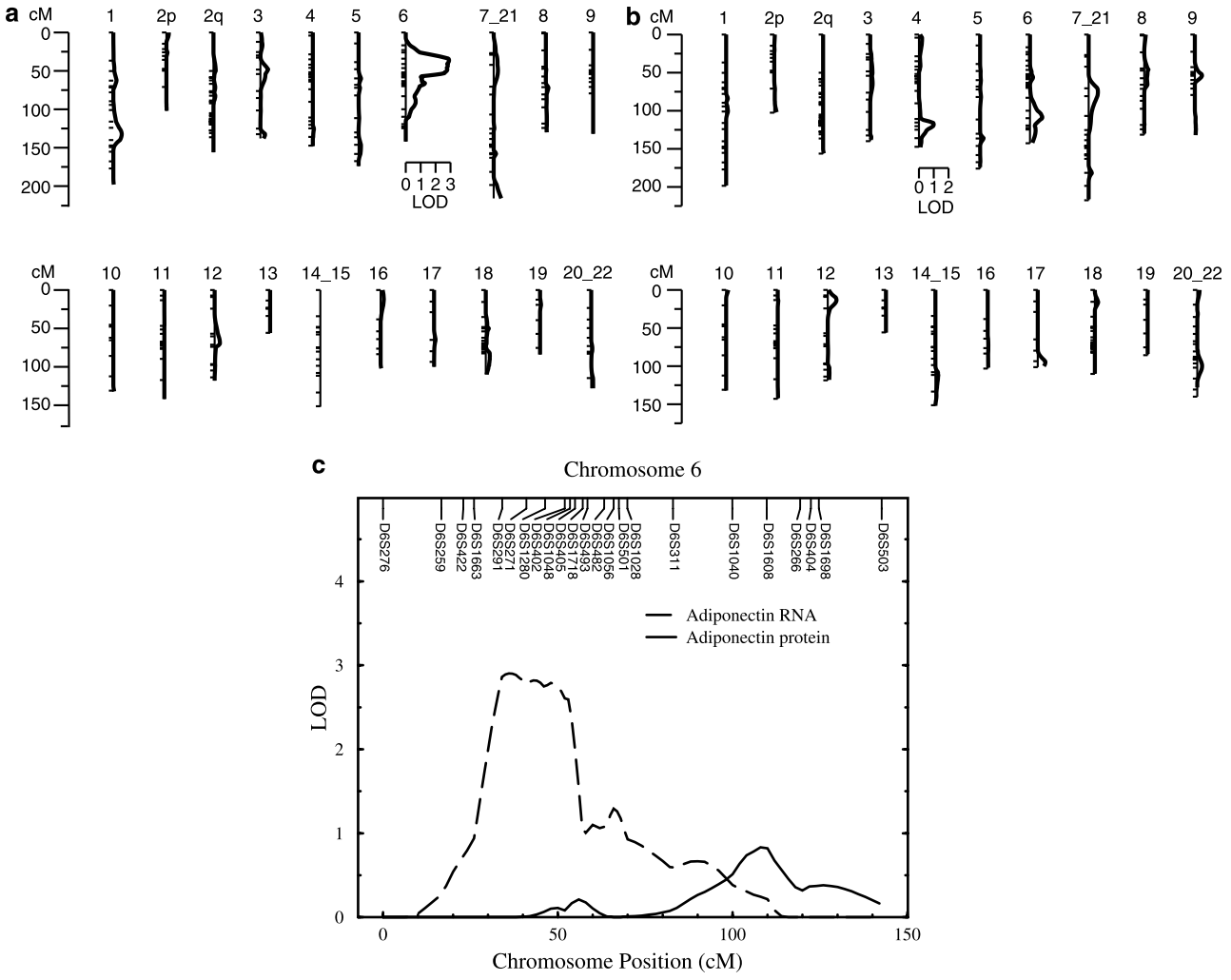

Figure 3 (**a**) Adiponectin mRNA genome-wide scan. Numbers show human orthologous regions. (**b**) Adiponectin circulating protein genome-wide scan. Numbers show human orthologous regions. (**c**) Signals on chromosome 6p21 for adiponectin mRNA and protein levels.

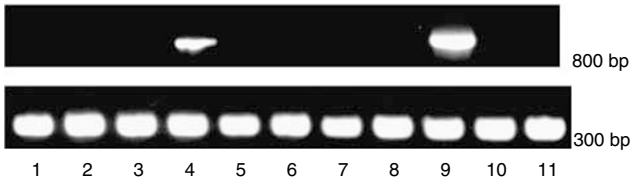

Figure 4 Expression of adiponectin mRNA across tissues in baboons. Tissue-specific expression of baboon adiponectin mRNA (upper panel) and 18S ribosomal subfraction (lower panel). (1) Stomach fundus, (2) ovary, (3) colon, (4) subcutaneous adipose tissue, (5) small intestine, (6) hypothalamus, (7) placenta, (8) monocytes, (9) omental adipose tissue, (10) pancreas and (11) liver. Agarose gel (2%) stained with ethidium bromide was used. A volume of 5 µl of PCR reaction was loaded per well.

an inverse association between adiponectin levels, glucose tolerance, adiposity and lipid metabolism (Fruebis *et al*., 2001; Hotta *et al*., 2001; Esposito *et al*., 2003; Kershaw and Flier, 2004; Shimada *et al*., 2004). The present investigation provides information on the genetic component present within these associations.

The ρ_G value of the genetic correlation of adiponectin mRNA and adipocyte volume is not statistically significant; however, the direction and magnitude of the estimated genetic correlation support the correlation observed with adiponectin protein levels. These data indicate the possible exertion of pleiotropic effects on adiponectin expression and adipose cell volume. The increase in adipocyte volume has been related to upregulation of the expression of pro-inflammatory cytokines (Garaulet *et al*., 2000; Weyer *et al*., 2000; Heilbronn *et al*., 2004; Rotter Sopasakis *et al*., 2004; Pausova, 2006), insulin resistance and the risk for type 2 diabetes (Weyer *et al*., 2000). It has been proposed that this relationship could be mediated by changes in intracellular signaling affecting regulation of gene expression (Weyer *et al*., 2000; Pausova, 2006). Enlargement of adipocytes may be caused by less proliferation and differentiation of these cells and may be accompanied by deposit of fat in nonadipose tissues (Heilbronn *et al*., 2004). A negative genetic correlation between adiponectin protein and triglycerides has been observed in human family studies in Hispanic children

($\rho_G = -0.24$, $P < 0.05$) (Butte *et al*., 2005) and adults ($\rho_G = -0.35$, $P < 0.05$) (Comuzzie *et al*., 2007), indicating the presence of pleiotropy. The genetic correlations in baboons were higher than those reported in studies of human families and we considered that the homogeneity in diet and environmental factors may contribute to the larger pleiotropic genetic effects in this species.

While no significant evidence of linkage was found for baboon adiponectin protein in the present investigation, results from the genome-wide scan indicate that a QTL influencing mRNA levels maps to an area on baboon chromosome 4p between microsatellite marker loci D6S422 and D6S1718, which is orthologous to the human 6p21 region. This same region has been linked to insulin resistance and type 2 diabetes traits in human studies (Norman *et al*., 1995; Luo *et al*., 2001; An *et al*., 2005). Recently, An *et al*. (2005) reported QTL in this region for parameters of glucose metabolism in response to exercise training in Whites and Blacks. A study by Norman *et al*. (1995) identified significant evidence for a QTL influencing variation in percentage of body fat in a sib-pair study in Pima Indians. To our knowledge, this is the first linkage analysis using adiponectin mRNA expression as a quantitative phenotype.

A positional candidate in this region, based on the human map, is the tumor necrosis factor-α (TNFα, GI: 89161210), which is a well-characterized adipokine associated with obesity-induced insulin resistance, increased adipocyte volume and regulation of the expression of adiponectin (Bruun *et al*., 2003). There are four human and two rodent studies showing association between genetic variations in tumor necrosis factor-α and adiposity-related phenotypes (body mass index, percentage of body fat, waist circumference) (Hanson *et al*., 1998). One human study reported an association between the −308G/A single nucleotide polymorphism in the tumor necrosis factor-α gene and expression of adiponectin (Rankinen *et al*., 2006). Other positional candidates in this chromosomal region include the peroxisome proliferator-activated receptor-δ (PPAR-δ, GI: 89886454) and glucagon-like peptide receptor 1 (GLP1R, GI: 402480).

In summary, the sequence and tissue-specific expression of baboon adiponectin showed a high degree of similarity to human. Our results confirmed a genetic contribution to the variation of adiponectin protein levels in blood and the presence of common genes influencing adiponectin levels, triglycerides and body mass, as observed in human studies. The present study provided novel findings suggesting the existence of genes controlling adiponectin expression and cell volume and other phenotypes related to obesity and insulin resistance. In addition, our investigation identified a new QTL linked to adiponectin mRNA expression. This QTL harbors some positional candidates that have previously been proposed using diabetes-related traits in human studies.

Acknowledgements

This work was supported by NIH Grants HL28972, RR3986 and MH59490.This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant no. C06 RR13556-01 from the National Center for Research Resources, NIH.

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